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Likelihood ratio statistics for DNA mixtures allowing for drop-out and drop-in

Adele A. Mitchell, Jeannie Tamariz, Kathleen O'Connell, Nubia Ducasse, Mechthild Prinz, Theresa Caragine *

Office of Chief Medical Examiner of the City of New York, Department of Forensic Biology, New York, NY, USA

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ABSTRACT

The likelihood ratio (LR) is the recommended approach for forensic DNA mixture analysis by the DNA commission of the ISFG, as it makes maximum use of available data and parameters for allelic drop-out and drop-in can be incorporated. We have developed and validated a LR method and software for analysis of mixed evidence samples in which drop-out of true contributors' alleles and drop-in of extraneous alleles may have occurred. This method, the forensic statistical tool (FST), employs empirically determined drop-out and drop-in probabilities for single source samples and mixtures. The LR is computed for pairs of prosecution and defense hypotheses based on sample characteristics specified by the user. Data from up to three evidence amplifications may be considered simultaneously. The performance of the program was evaluated with hundreds of profiles generated from blood and buccal samples, purposefully degraded samples, and touched items with one, two, three, and four known contributors. The validation demonstrated that FST assigns an appropriate weight to all types of comparisons. FST is now being used in casework and results continue to be consistent with qualitative assessments.

1. Introduction

The likelihood ratio (LR) is the method recommended by the DNA Commission of the International Society of Forensic Genetics (ISFG) for analysis and interpretation of forensic DNA mixtures [1]. Although the LR framework can accommodate allelic drop-out and drop-in [2], the standard formulation of the LR does not include parameters for these phenomena [3]. That is, as the LR is generally applied, the alleles found in the mixture must be fully explained by the contributors conditioned upon in the numerator and the denominator.

The Office of Chief Medical Examiner of the city of New York (OCME) has developed and validated a LR method that allows for allelic drop-out and drop-in (manuscript in preparation). The method is implemented in a computer program known as the forensic statistical tool (FST). FST is the only available program that simultaneously considers up to three replicates of a DNA sample, which is particularly useful for samples with small amounts of template DNA. The probability of drop-out used by FST is a function of the amount of template DNA amplified per reaction, the number of contributors to the mixture, the estimated mixture ratio (similar or dissimilar), and the Identifiler® (Applied Biosystems, Foster City, CA) kit. The probability of drop-in is a function of the number of amplification cycles. OCME uses 28 cycles (standard) for samples which are greater than 100 pg of template DNA per reaction and 31 cycles (high sensitivity) for samples which are less than 100 pg of template DNA. Samples with 100 pg can be amplified with either 28 or 31 cycles, depending on the sample type. FST users must specify the hypotheses, the estimated mixture ratio, amount of template DNA, and whether 28 or 31 cycles were used.

2. Validation design and overview of results

To determine drop-out and drop-in rates, over 2000 samples representing a wide range of DNA concentrations and mixture types were evaluated using both standard and high sensitivity STR typing methods. The finished program incorporating these rates was tested with 439 samples. These included purposefully degraded and non-degraded mixtures with known contributors, as well as mock casework samples from items handled by two, three, or four known persons. The resulting mixtures contained various DNA concentrations and ratios of contributors. Forty-one samples were evaluated twice with different mixture ratio specifications for a total of 480 test runs.

Samples were processed and interpreted manually according to current OCME casework protocols. Qualitative comparisons were made to known references. Contributors could be possibly

* Corresponding author. Tel.: +1 212 323 1250; fax: +1 212 323 1590.
E-mail address: tcaragine@ocme.nyc.gov (T. Caragine).

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associated to a mixture if all of the alleles consistent with their profile were labeled in the mixture, or if the absence of some of these alleles could be scientifically explained. In some cases, no conclusions could be drawn regarding whether an individual contributed to the mixture. FST was then used to calculate the LR for each comparison. Overall, LR results were consistent with manual comparisons for all sample types. Most associations gave positive log LRs, while exclusions generated negative log LRs. For samples wherein no manual conclusion could be drawn, log LRs were either positive or negative. The strength of the result was evident in the magnitude of the log LR.

In addition, all samples were compared to a database of 1246 DNA profiles of anonymous individuals that did not contribute to these mixtures. This generated 557,874 comparisons to non-contributors from over 480 runs. This experiment was designed to test the separation between contributors and non-contributors, as well as to evaluate possible chance of positive associations. Due to allelic sharing, a small number of non-contributors, 0.08%, generated positive log LRs. Based on these data, we can predict how often a particular LR is expected to be observed in a population of non-contributors. This prediction can provide meaningful context for an LR value.

3. Discussion and case examples

On July 1, 2011, OCME began using FST for forensic casework samples. Using the two- or three-person mixture module, FST is applied to mixtures for which a comparison profile, such as that from a suspect, is available and almost all of the alleles seen in the comparison profile are labeled in the mixture. Depending on the circumstances of the case, FST calculations can also be performed for mixtures involving an association to a victim or other individual. For intimate samples or certain case scenarios, a victim or other individual may be used as a known contributor to a mixture in the formulation of the LR hypotheses. One challenge in the application of FST to casework samples is the selection of appropriate prosecution and defense hypotheses. Assuming another individual within the case as a known contributor may only be uncontested for intimate samples. This is especially relevant for cases with multiple suspects. In such situations where another individual within a case can be associated to the mixture and that connection is informative, OCME decided to report both sets of numbers. Another option in this type of case would be to assume a known contributor in the numerator only, and to use unknown individuals in the denominator, thereby presenting the strength of the evidence for inclusion of both suspects together.

Two recent cases illustrate the type of LR values obtained using FST. For the first case, two people (one male and one female) were associated with a mixture found on a marijuana blunt connected to an assault case. All of the alleles seen in the DNA profile of the female suspect were labeled in the mixture. Not all but four of the alleles consistent with those of the male suspect's DNA profile were labeled, and some unlabeled alleles were visible. When constructing LRs in this case, one important question arises that whether or not to include each individual suspect as a known contributor to the mixture while testing the other suspect. If the female is included as a known contributor while testing the male suspect using the three-person mode with dissimilar ratios, the LR is 578. If she is not included, the LR is 222. If the male is included as a known contributor to the mixture while testing the female suspect, the LR is 2.36×10^9. If he is not included, the LR is 9.58×10^8. Interestingly, in spite of the large difference in LRs of the two suspects, the other suspect being a known contributor increased the LR approximately to two-fold for each one of them. The overall difference in the LR values between the two suspects illustrates the effect of the four drop-out events that were invoked for the male suspect.

A second case involves a shooting. A tip led police to a car from which three guns were recovered, and five suspects were subsequently arrested. Swabs from two different guns were sampled with high sensitivity testing. Suspect 1 was associated to a mixture detected on the trigger of one gun. Not all but two alleles consistent with his profile were labeled in the mixture. Testing suspect 1 using the two-person module with similar ratios generated a LR of 5730. No conclusions could be drawn regarding whether suspect 2 contributed to the mixture found on the slide release from the second gun. Four alleles consistent with his profile were not detected in this mixture. Furthermore, using the two-person module with similar ratios, the resulting LR was 0.3 which did not provide him support for contributing to this sample. This LR value reflects the four drop-out events invoked over the three replicates of this mixture. For case two, invoking drop-out had a stronger effect than in the previous case example, which illustrates the different scenarios employed with FST. The expected drop-out of a minor contributor to a three-person mixture with dissimilar ratios, as seen in the first case example, is higher than that of one of the contributors to a two-person mixture with similar ratios, as observed in the second case. These differences highlight the importance of determining the number of contributors to a mixture for both high and low template DNA samples [4,5].

Conflict of interest

None.

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